Pathogenicity of Entomopathogenic Fungi *Metarhizium anisopliae* (METSCH) SOROKIN and *Metarhizium brunneum* (PETCH) against Drywood Termites (*Cryptotermes* sp.) (Isoptera: Kalotermitidae)

by

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Abstract

Pathogenicity bioassay of entomopathogenic fungi *Metarhizium anisopliae* and *M. brunneum,* with 10^5, 5.10^5, 10^6, 5.10^6, and 10^7conidia/ml against drywood termites have been conducted. Result shows that conidia concentration of both entomopathogenic fungi had effect whereas kind of fungi had no effect on drywood termite mortality. Drywood termites mortality increased as increasing of both fungi conidia concentration. On 10^6 conidia/ml concentration both of fungi could cause drywood termite mortality more than 60% whereas on 10^7 conidia/ml more than 90%, mortality of drywood termite applied by *M. anisopliae* and *M. brunneum* 93.94% and 96.97% respectively. Even though there was no difference between *M. anisopliae* and *M. brunneum* pathogenicity, nevertheless from LC and $LT_{25,\ 50}$ and $_{95}$ *M. brunneum* showed better than *M. anisopliae*. *M. brunneum* had lower LC dan $LT_{25,\ 50}$ and $_{95}$ value than *M. anisopliae*.

Key words: Patogenicity, Entomopathogenic Fungi, *Metarhizium anisopliae*, *Metarhizium brunneum*, Drywood Termites.

Introduction

Drywood termites are tropic and sub tropic insects that used wood as its' main food. The ability of termites in consuming wood put termites as very dangerous pest especially in Indonesia (Tarumingkeng, 2004). Since known as very dangerous pest, an effort to control drywood termites, *Cryptotermes* spp. (Isoptera: Kalotermitidae) will always go on, especially using microbial insecticide. Oka (1995) stated that using microbial insecticide for controlling termites has several advantages such as has relative low cost, have many strains and can be germinate strain *invitro*. Setiawati (2004 cit. Yoza, 2007) suggested that using microbial insecticide in controlling termites besides has relative low cost, those agents relative have no negative effect for human and environment. Currently, the microbial insecticide used for termites control in the world is primarily fungi.

Fungi are potential pathogenic agents in controlling termites (Culliney et al., 2000). The ability of fungi in controlling termites according to Grace et al., (1992) supported by fungi characteristics that has a slow-acting nature similar to that of successful chemicals, the ability to self-replicate and the ability of fungal spores to spread by termite social behavior. Kramm et al. (1982) found that

conditions in a termite nest, moderate temperature and high humidity, are also conducive to the growth of fungal species and are important factors in fungal survivability and propagation.

Milner et al. (1996) review a wide variety of fungal pathogens that been reported as potential pathogens to termites. Stamet (2003) found several genera entomopathogenic fungi including Metarhizium, Beauveria, Paecilomyces, Hirsutella, Verticillium and other fungi Imperfecti, the Entomophthoracae and other Phycomycetes, and sexually reproducing fungi such as Cordyceps and other Ascomycetes. Among all of entomopathogenic genus, Metarhizium is one of entomopathogenic fungi that very potential in controlling termites especially *M. anisopliae* and *M. brunneum*. Both of these entomopathogenic fungi have various pathogenicity.

Even though Prayogo, *et al*. (2005) stated that *M. anisopliae* had been used to control insect for along time, Boucias and Pendland (1998) found that *M. anisopliae* had infected more than 200 species of insects, and Strack (2003 cit. Prayogo, 2006) said that *M. anisopliae* most effective if used in controlling insect in Isoptera Order, Desyanti (2007) found that viability of *M. anisopliae* lower than *M. brunneum* and supposed influence it's pathogenicity. According to variety of metarhizium patogenicity and it's ability in controlling termites, it is important to know wich one is better.

Materials and methods

Entomopathogenic fungi (*M. anisopliae* dan *M. brunneum)* originated from Insect Pathology Laboratory, Pest and Diseases Department, Bogor Agricultural Institute. Before using as microbial insecticide, those fungi were regenerated at termite body, cultured in PDA medium, and incubated in room temperature. Fungus from PDA medium isolated until forming pure isolates. Pure isolates of fungi then cultured in PDA medium and incubated for 3 weeks. Conidial suspension of fungi got out from PDA medium through shake down the petridish use sterile aquades that contain 0.05% Tween 80. After that those suspension poured into test tube. Serial dilution method used for making suspension to the needs of (10^5, 5.10^5, 10^6, 5.10^6, and 10^7conidia/ml). Conidial density of fungi counted using haemocytometer.

Pathogenicity test of fungi use drywood termites that collected from infested building in Padang. Pathogenicity test of both fungi applied through topical method to 1 soldier and 10 worker termites. Infected termites transferred in to plastic bowl that covered with tissue paper which used as food of termites. Infested termites kept in dark room. Mortality of termites counted everyday for a week. Isolate be said to be pathogenic if can cause mortality > 60% (Desyanti, 2007). Value LC and LT $_{25, 50}$ and $_{95}$ of both fungi analysed using probit analysis (Finney, 1971).

Result and discussion

Pathogenicity testing of *M. anisopliae* and *M brunneum* against drywood termites showed that both of fungi have same pathogenicity level. It's supposed because of those fungi originated from the same genera. Defrita (2008) found that entomopathogenic fungus that originated from the same genus (*Aspergilus niger* and *Aspergilus* sp.) had no different pathogenicity level against drywood termites.

Table 1 also showed that conidial concentration of entomopatogenic fungi *M. anisopliae* and *M. brunneum* have differed pathogenicity against drywood termites, the higher conidial

concentration of fungi, the higher mortality of termites. The increase of mortality of termites as concentration increase supposed related to more and more amount of fungi conidial that infect those insects. Prayogo, et al. (2005) stated that the more conidial fungi concentration applied to insect, the more insect mortality. This condition run the chance of conidial to adhere at termites cuticle. This researh in the respect of Nopyanti et al., (2009) research that found concentation of annona extract influence mortality of drywood termites.

Table 1: Mortality of drywood termites after applying entomopathogenic fungi *M. anisopliae* and *M. brunneum* (%)

A Factor (kind of fungi)	B Factor (Conidial fungi consentration)					
	B0	B1	B2	B3	B4	B5
A1	15,15a	36,36b	51,51c	63,63d	81,82c	93,94f
A2	12,12a	42,42b	57,57c	69,69d	84,85e	96,97f

Note: Numbers that followed by the same lowercase indicate that there is no significant different on 5% significant level.

A1 = *M. Anisopliae*
A2 = *M. brunneum*
B0 = control
B1 = 10^5 conidia/ml
B2 = 5.10^5 conidia/ml
B3 = 10^6 conidia/ml
B4 = 5.10^6 conidia/ml
B5 = 10^7 conidia/ml

Research result also showed that both of entomopatogenic fungi had high patogenicity and effective in controlling drywood termite (Figure 1). Data showed that with 10^6 conidia/ml concentration both of fungi had already controlled drywood termites more than 60%, whereas in 10^7 conidia/ml concentration both of fungi could kill drywood termites more than 90%. Desyanti (2007) stated that one organism could be said as pathogenic if could infected and caused mortality in pest while one of biological control agents could be said effective if could kill more than 60% of pest.

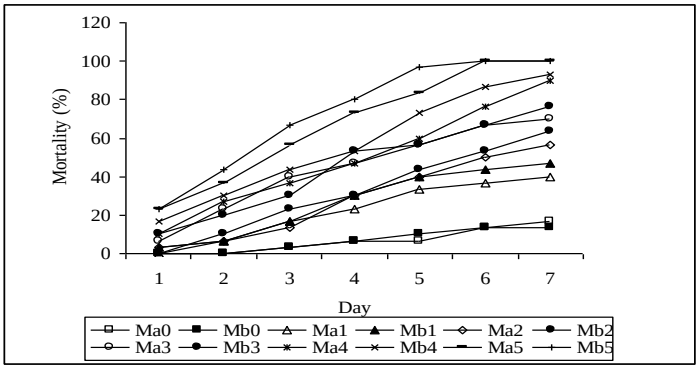

Figure 1 Drywood Termites Mortality Rate after Application of Entomopathogenic Fungi *M. anisopliae* and *M. brunneum*

Ma = *M. Anisopliae*
Mb = *M. brunneum*
0 = control
1 = 10^5 conidia/ml
2 = 5.10^5 conidia/ml
3 = 10^6 conidia/ml
4 = 5.10^6 conidia/ml
5 = 10^7 conidia/ml

Milner et al (1996) stated that fungi are the most promising entomopathogenic for the development of a microbiological termiticide. It means that both of metharhizium fungi could be an alternative biotermiticides. Neves et al. (2004) stated pathogenicity of entomopathogenic fungi determined by several factors including host defense and fungi physiology such as viability, growth rate, spore formation ability and ability to produce enzyme and toxin, and environment. Neves and Alves (2004, cit. Desyanti et al., 2007) stated that insect mortality influenced by application dose and virulence of fungi. Butt et al.,(2001 cit. Suharjono, 2009) stated that pathogenicity level of fungi influenced by several pactors including insect phisiology, such as host defence mechanism and fungi pgisiology such as viability, growth rate, sporulate ability and secunder metabolit (enzime and toxin) and environment factors.

Although there was no differentiation of pathogenicity of both fungi in causing termites mortality, in Figure 1 could be seen that entomopathogenic *M. brunneum* had higher ability in controlling drywood termites. Conidial concentration of *M. brunneum* that can cause 25, 50 and 95% of mortality of drywood termites also lower than *M. anisopliae* (Table 2). The better ability of entomopathogenic fungi *M. brunneum* in controlling drywood termites is supported by Ginting (2008) that found pathogenicity of *M. brunneum* against *Coptotermes curvignathus* and *Schedorhinotermes javanicus* higher than *M. anisopliae*. Desyanti, et al., (2007) also found that *M. brunneum* is the most effective fungi as bio control agents for soil termites *Coptotermes gestroi* because of its' highest pathogenicity.

Table 2: $LC_{25, 50}$ and $_{95}$ of *M. anisopliae* and *M. brunneum* fungi against drywood termites

Kind of fungi	Probability	95 Percent Fiducial Concentration		
			Lower	Upper
M. anisopliae	0,25	$2,6 \times 10^4$	_	_
	0,50	$4,8 \times 10^4$	_	_
	0,95	$10,1 \times 10^4$	_	_
M. brunneum	0,25	$1,5 \times 10^4$	_	_
	0,50	$3,9 \times 10^4$	_	_
	0,95	$9,9 \times 10^4$	_	_

The ability of entomopathogenic fungi *M. brunneum* in controlling drywood termites also showed in $_{25, 50}$ and $_{95}$ LT value (Table 3). It's showed that the ability of this fungi to be related to it's lethal time. *M. brunneum* had lower LT $_{25, 50}$ and $_{95}$ value than *M. anisopliae*. It means that entomopathogenic fungi *M. brunneum* more effective than *M. anisopliae*.

Table 3 $LT_{25, 50, 95}$ of *M. anisopliae* and *M. brunneum* fungi against drywood termites

Kind of fungi	Probability	95 Percent Fiducial Time		
			Lower	Upper
M. anisopliae	0,25	0,34584	0,17133	0,43493
	0,50	0,51449	0,42161	0,57434
	0,95	0,92578	0,83003	1,11621
M. brunneum	0,25	0,25410	0,08391	0,34804
	0,50	0,42822	0,32948	0,49258
	0,95	0,85284	0,76596	1,00744

The lower LT and $LC_{25, 50, 95}$ value of *M. brunneum* than *M. anisopliae* showed that although both of fungi have same pathogenicity but concentration and time needed by *M. brunneum* to control drywood termites lower than *M. anisopliae*. The difference of fungi pathogenicity indicated that each of fungi has specific potency in controlling drywood termites. Stamet (2003) states that biological control agents had tried with varying results. Fungal control agents are promising group of insect pathogens suitable for use as bio pesticides for the control of insects. However, limited availability, cost and reliability have hampered the development of such fungal control agents. Host range and specificity has been a problem as well as an advantage; a fungal pathogen that is virulent and pathogenic to one insect species may be ineffective against other species, even those of the same genus. However, some success had demonstrated.

Conclusion

Conidial concentration of entomopathogenic fungi *M anisopliae* and *M. brunneum* had effect whereas kind of fungi had no effect on drywood termite mortality. Drywood termite mortality increased as increasing of both fungi conidia concentration. Even though there was no differences pathogenicity between both fungi, nevertheless from LC and $LT_{25, 50}$ and $_{95}$ appearance, *M. brunneum* showed better than *M. anisopliae*. *M. brunneum.*

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